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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

TXR#: 0052841

DATE: April 7, 2005

SUBJECT: Ethylene Oxide: Review of Acute Neurotoxic and Subchronic Neurotoxicity Studies (OPPTS 870.6200; OPP §81-8) in Rats

FROM: Santhini Ramasamy, PhD, DABT, MPH
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Santhini Ramasamy 4.7.05

TO: Susan Bartow
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THRU: Susan V. Hummel , Branch Senior Scientist
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TASK ID: DP Code: D235475
P.C. Code: 042301

REGISTRANT: Allied Signal, Inc., Morristown, NJ and ARC Chemical Division, Balchem Corporation, Slate Hill, NY

Action Requested: Review of Acute Neurotoxic Study (MRID 44256402) and Subchronic Neurotoxicity studies (MRID 44359401; MRID 44256401).

Agency's Response:

Both acute inhalation neurotoxicity and subchronic neurotoxic studies are found Acceptable and satisfies the guideline requirements (870.6200 or OPP §81-8). The conclusions of the neurotoxicity studies are summarized below.

Acute Neurotoxicity Study

In an acute neurotoxicity study (MRID 44256402), groups of ten Sprague-Dawley rats/sex were exposed to 0, 100, 300, or 500 ppm Ethylene Oxide for six hours by whole body inhalation and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on all animals/sex/group immediately following exposure on day 1 and on days 8 and 15. At study termination, five animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, all control and high-concentration animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations. All animals survived to scheduled termination. For males and females, absolute body weight was similar between the treated and control groups throughout the study. Body weight gain was slightly decreased for the high-concentration groups during the 2-week observation interval. Overall weight gain by the high-concentration males and females was 88% and 86%, respectively, of the control level. Food consumption was not affected by treatment.

During FOB assessments after exposure, an increased number of high-concentration males and females had drooping eyelids or half-closed eyes (5 and 3, respectively), a low arousal level (9 and 6, respectively), and no response to an approaching object (6 and 4, respectively) compared with 0-1 in the control groups. In the mid-concentration groups, increased incidences were observed for low arousal (5) and no approach response (6) in males and of drooping eyelids (5) in females. Slightly impaired locomotion was observed in two mid-concentration males, and in one and two high-concentration males and females, respectively, on day 1. In mid- and high-concentration males, some evidence of persistent effects were observed on day 8 and 15. On day 8 in the control, low-, mid-, and high-concentration males, slightly impaired locomotion was seen in 1, 2, 5, and 4 animals, respectively, and low arousal was seen in 1, 3, 5, and 4, respectively. On day 15 in the control, low-, mid-, and high-concentration males, slightly impaired locomotion was observed in 1, 3, 5, and 7 animals, respectively, and low arousal was observed in 0, 3, 6, and 6, respectively. No effects were found on fore- and hind-limb grip strengths, landing foot splay, or reflex assessments.

Motor activity was markedly lower for high-concentration males and females compared with controls after exposure on day 1. Total activity on day 1 for high-concentration males and females was 50% and 55%, respectively, of the control group level. Activity by the mid-concentration males was 50% of the control level during the first 5-minute interval resulting in total activity 70% of the controls for the entire session. No other treatment-related effects were noted.

Gross necropsy was unremarkable. No microscopic lesions were described for the brain, spinal column, or peripheral nerves from any control or high-concentration rat.

The acute inhalation neurotoxicity LOAEL for ethylene oxide in Sprague-Dawley rats is 300 ppm based on FOB findings in males and females and impaired locomotion and reduced motor activity in males. The NOAEL is 100 ppm.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. In the compliance statement it was noted that the study was not conducted according to GLP standards because an archival sample of each lot of test material was not taken as required.

Subchronic Neurotoxicity Studies

In a range-finding study (MRID 44256401), groups of five Sprague-Dawley rats/sex were exposed to target concentrations of 0, 100, 300, 400, or 500 ppm Ethylene Oxide for six hours/day, five days/week for 4 weeks by whole body inhalation. The study was designed to assess the inhalation toxicity of ethylene oxide and to establish exposure concentrations for longer-term studies. Mean analytical exposure concentrations were 0, 98.4 ± 3.76, 300 ± 15, 397 ± 23, and 501 ±12 ppm. The mean nominal concentrations were 0, 109 ± 10.7, 292 ± 19, 382, ± 24, and 506 ± 11 ppm. Particle size distribution measurements suggested the absence of any aerosol formation.

One 500 ppm female was found dead on day 18. Antemortem clinical signs included irregular gait, labored breathing, paleness, lethargy, emaciation, anogenital stains, black/brown stains on the snout, unthrifty coat, decreased food consumption, and decreased fecal volume/no stool. All other rats survived to terminal sacrifice. Irregular gait and decreased fecal volume were observed in all animals exposed to 500 ppm. Some animals in the 500 ppm group also exhibited lethargy, prostration, emaciation, yellow angenital staining, moist rales, labored breathing, black/brown stains on the snout, paleness, emaciation, and decreased food consumption.

Decreased ($p \leq 0.05$ or 0.01) mean body weight and body weight gain were observed in animals exposed to 300, 400, or 500 ppm. At week 4, mean body weight was decreased 12.1%, 23.8%, and 42.4% in males and 14.4%, 20.1%, and 40.6% in females in the 300, 400, and 500 ppm groups, respectively. Food consumption was decreased ($p \leq 0.01$) in 500 ppm males in weeks 1 (18%) and 3 (12%) and in 500 ppm females in week 1 (15%).

Hindlimb grip strength was decreased ($p \leq 0.05$ or 0.01) at 300, 400, and 500 ppm in both males and females at weeks 3 and 4 in all trials. In the 500 ppm group, decreases averaged 53% and 60% at week 3 and 76% and 86% at week 4 for males and females, respectively. In the 400 ppm group, decreases averaged 31% in males and 38% in females at week 3 and 48% for both sexes at week 4. In the 300 ppm group, decreases averaged 27% in males and 23% in females at week 3 and 36% for males and 22% for females at week 4. Landing foot splay was also decreased

($p \leq 0.05$ or 0.01) in both sexes at weeks 3 and 4 in the 400 and 500 ppm groups. In the 500 ppm group, decreases averaged 65% and 64% at week 3 and 68% and 72% at week 4 for males and females, respectively. In the 400 ppm group, decreases averaged 33% in males and 31% in females at week 3 and 42% for males and 29% for females at week 4.

Decreased absolute brain weight was noted in males exposed to 400 (6.8%; $p \leq 0.05$) or 500 ppm (8.9%; $p \leq 0.01$). No brain weight effects were noted in any females or in males exposed to 100 or 300 ppm. No treatment-related macroscopic lesions were noted at necropsy. Treatment-related microscopic lesions were noted in all males and females from the 500 ppm group and included minimal to slight vacuolation of the white matter of the thalamus and medulla oblongata. No similar microscopic lesions were noted in the control animals.

Based on the results of this study, the NOAEL for ethylene oxide administered to Sprague-Dawley rats via whole body inhalation for 4-weeks is 100 ppm. The LOAEL is 300 ppm based on decreased body weight and body weight gain and decreased hindlimb grip strength.

In a subchronic neurotoxicity study (MRID 44359401), groups of fifteen Sprague-Dawley rats/sex were exposed to 0, 25, 50, 100, or 200 ppm Ethylene Oxide for six hours/day, five days/week for 14 weeks (at least 65 exposures) by whole body inhalation. Exposure concentrations were selected based on results of a range-finding study, MRID 44256401. To assess the reversibility of any observed effects, ten rats/sex/dose were observed during an additional 13-week recovery period. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on ten animals/sex/group pre-test, and during the exposure period (weeks 5, 9, and 14), and after the recovery period (weeks 27/28). Following at least 65 exposures, five animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Also, after the 13-week recovery period, five animals/sex/group were sacrificed and selected tissues preserved for neuropathological examination. The remaining five animals/sex/group were sacrificed and discarded. Of the perfused animals, all control and high-concentration animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations. There were no deaths during the exposure period in any test group. One male exposed to 100 ppm was found dead during the recovery period (4 weeks after termination of exposure). Antemortem observations included lethargy, paleness, labored breathing, ano-genital staining, decreased fecal volume/no stool, and decreased food consumption.

Mean body weight was decreased approximately 8-13% ($p \leq 0.05$ or 0.01) in high-concentration males from week four until cessation of exposure in week 13. Mean body weight gain was decreased ($p \leq 0.05$ or 0.01) in high-concentration males during every week of the exposure period except week three; the decrease over weeks 1-13 was 16%. In high-concentration females, mean body weight was decreased approximately 8-10% ($p \leq 0.05$) from weeks 10 through 13, and mean body weight gain was decreased approximately 9% from weeks 6 through 13. The effects on body weight were reversible. The high-concentration males and females gained more weight than controls during the recovery period, and at week 26 mean body weight of high-concentration males was decreased only 3% compared to controls, and body weight gain of high-concentration females was increased 3.5% compared to controls. There were no treatment-related effects on body weight or body weight gain in either sex in the 25, 50, or 100 ppm groups. The only treatment-related effect on food consumption was an increase ($p \leq 0.05$) in food consumption in high-concentration males and females during the recovery period.

A 25% decrease ($p \leq 0.05$) in hindlimb grip strength in high-concentration females at the end of the exposure period was noted in the FOB assessment. No other treatment-related FOB effects were noted in either sex at any time point at any test concentration. There were no treatment-related effects on motor activity.

A malignant glioma was noted in the cerebral cortex of one high-concentration male following the recovery period. Mononuclear cell leukemia was noted in the male exposed to 100 ppm that died during week 4 of the recovery period. A hemangiosarcoma in the skin/subcutaneous tissue was noted in one 25 ppm male, and basal cell carcinoma of the skin was noted in one 50 ppm female and one 200 ppm female. Although there was no concentration-response relationship or common tumor type, these neoplasms were considered treatment-related because of the atypical presence of neoplasms in animals of this age and the lack of neoplasms in the control group.

Based on the results of this study, the NOAEL for ethylene oxide administered to Sprague-Dawley rats via whole body inhalation for 13-weeks (at least 65 exposures) is 100 ppm. The LOAEL is 200 ppm based on decreased body weight and body weight gain in males and females and decreased hindlimb grip strength in females.

Based on the data presented in this study, there is evidence of carcinogenicity in Sprague-Dawley rats exposed to ethylene oxide by inhalation during the thirteen week recovery period following at least 65 exposures.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. In the compliance statement it was noted that the study was not conducted according to GLP standards because empty test material cylinders were returned to the Sponsor during the course of the study to be refilled with test material and were not retained for

the duration of the study, and because an archival sample of each lot of test material was not taken as required.

DATA EVALUATION RECORD

ETHYLENE OXIDE/042301
[OPPTS 870-6200a (§81-8)]

STUDY TYPE: ACUTE INHALATION NEUROTOXICITY STUDY IN RATS
MRID 44256402

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 79-2005

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Reregistration Branch 4, Health Effects Division (7509C) **Date** 3/22/05
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Registration Action Branch 3, Health Effects Division (7509C) **Date** 3/22/05

TXR#: 0052841

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats [OPPTS 870.6200a (§81-8)] OECD 424.

PC CODE: 042301 **DP BARCODE:** D235475
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Ethylene oxide (0.484-0.520%)

SYNONYMS: none

CITATION: Mandella, R.C. (1997) An acute inhalation neurotoxicity study of ethylene oxide (498-95A) in the rat via whole-body exposure. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study number 95-6097, March 21, 1997. MRID 44256402. Unpublished.

SPONSOR: AlliedSignal, Inc., 101 Columbia Road, Morristown, NJ 07962-1139 and ARC Chemical Division, Balchem Corporation, P.O. Box 180, Slate Hill, NY 10973

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 44256402), groups of ten Sprague-Dawley rats/sex were exposed to 0, 100, 300, or 500 ppm Ethylene Oxide for six hours by whole body inhalation and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on all animals/sex/group immediately following exposure on day 1 and on days 8 and 15. At study termination, five animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, all control and high-concentration animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations. All animals survived to scheduled termination. For males and females, absolute body weight was similar between the treated and control groups throughout the study. Body weight gain was slightly decreased for the high-concentration groups during the 2-week observation interval. Overall weight gain by the high-concentration males and females was 88% and 86%, respectively, of the control level. Food consumption was not affected by treatment.

During FOB assessments after exposure, an increased number of high-concentration males and females had drooping eyelids or half-closed eyes (5 and 3, respectively), a low arousal level (9

and 6, respectively), and no response to an approaching object (6 and 4, respectively) compared with 0-1 in the control groups. In the mid-concentration groups, increased incidences were observed for low arousal (5) and no approach response (6) in males and of drooping eyelids (5) in females. Slightly impaired locomotion was observed in two mid-concentration males, and in one and two high-concentration males and females, respectively, on day 1. In mid- and high-concentration males, some evidence of persistent effects were observed on day 8 and 15. On day 8 in the control, low-, mid-, and high-concentration males, slightly impaired locomotion was seen in 1, 2, 5, and 4 animals, respectively, and low arousal was seen in 1, 3, 5, and 4, respectively. On day 15 in the control, low-, mid-, and high-concentration males, slightly impaired locomotion was observed in 1, 3, 5, and 7 animals, respectively, and low arousal was observed in 0, 3, 6, and 6, respectively. No effects were found on fore- and hind-limb grip strengths, landing foot splay, or reflex assessments.

Motor activity was markedly lower for high-concentration males and females compared with controls after exposure on day 1. Total activity on day 1 for high-concentration males and females was 50% and 55%, respectively, of the control group level. Activity by the mid-concentration males was 50% of the control level during the first 5-minute interval resulting in total activity 70% of the controls for the entire session. No other treatment-related effects were noted.

Gross necropsy was unremarkable. No microscopic lesions were described for the brain, spinal column, or peripheral nerves from any control or high-concentration rat.

The acute inhalation neurotoxicity LOAEL for ethylene oxide in Sprague-Dawley rats is 300 ppm based on FOB findings in males and females and impaired locomotion and reduced motor activity in males. The NOAEL is 100 ppm.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. In the compliance statement it was noted that the study was not conducted according to GLP standards because an archival sample of each lot of test material was not taken as required.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Ethylene oxide

Description:	colorless gas
Lot/Batch #:	a total of 22 cylinders were used; lot numbers are documented in the raw data
Purity:	0.484-0.520% a.i.
CAS # of TGAI:	75-21-8
Structure:	

2. **Vehicle and/or positive control:** Clean air was used as the carrier and negative control. No positive control was used in this study.

3. **Test animals:**

Species:	Rat	
Strain:	Sprague-Dawley CD® [Crl: CD® BR]	
Age/weight at dosing:	6 weeks; M: 141-158 g; F: 107-120 g	
Source:	Charles River Laboratories, Kingston, New York	
Housing:	Animals were housed individually in suspended, stainless steel, wire mesh cages.	
Diet:	Certified Rodent Diet, No. 5002 was available *ad libitum* except during exposure.	
Water:	Mains water was available *ad libitum* except during exposure.	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	37-94%
	Air changes:	not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	2 weeks	

B. STUDY DESIGN:

1. **In life dates:** Start: March 5, 1996; End: March 22, 1996

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 by a computerized random sort program so that body weight means were comparable. Animals were administered a single, 6-hour exposure in whole-body chambers and maintained for two weeks. Concentration levels were chosen by the sponsor. Exposure and neurobehavioral evaluations were staggered over four days with approximately equal numbers of animals/sex/group. A functional observational battery assessment followed by motor activity measurements were performed after exposure on day 1 and on days 8 and 15 of the post-exposure observation period. Survivors were sacrificed and a necropsy was performed.

TABLE 1. Study design				
Experimental Parameter	**Concentration (ppm)**			
	0	**100**	**300**	**500**
Total number of animals/sex/group	10	10	10	10
Behavioral testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	0/sex	0/sex	5/sex
Blood cholinesterase determination	0/sex	0/sex	0/sex	0/sex
Brain cholinesterase determination	0/sex	0/sex	0/sex	0/sex

3. **Test substance preparation and analysis**: The exposure chambers were operated dynamically under slight negative pressure. Animals were individually housed in wire mesh cages within the 1000 L chamber. The test article was delivered through a regulator equipped with inlet and outlet backpressure gauges from the cylinder. Flow controller meter settings were selected in order to achieve target concentrations. For all chambers the air flow rate was 201-210 Lpm, the time for complete air change was 5 min, and the equilibrium time was 22-23 minutes. Animals remained in the chamber for 15 minutes after exposure to allow the chamber to clear using clean air only. Samples were taken four times during exposure to measure concentration using gas chromatography. Nominal concentration was determined by dividing the test material volume consumed by the total volume of air used during exposure. Prior to the start of the study, samples from five locations within the chamber were taken to assess distribution of the test article within the chamber. Particle size distribution measurements were performed once during each exposure using a TSI Aerodynamic Particle Sizer. Results of chamber monitoring are given in Table 2.

TABLE 2. Results of exposure chamber monitoring				
Endpoint	**0 ppm**	**100 ppm**	**300 ppm**	**500 ppm**
Distribution (range of concentration, ppm)	NA	98.7-111	317-382	480-540
Mean analytical concentration (ppm)	ND	100 ± 3.8	307 ± 16	508 ± 29
Mean nominal concentration (ppm)	NA	89 ± 3.4	244 ± 0.6	468 ± 18
Particle size MMAD (μm) GSD Total mass conc. (mg/m^3)	 1.1 ± 0.34 2.0 0.00255 ± 0.00057	 1.4 ± 0.36 2.3 0.00289 ± 0.00072	 1.7 ± 0.54 2.3 0.00321 ± 0.00137	 1.6 ± 0.75 2.0 0.00254 ± 0.00017

Data taken from Appendix B, pp. 57-61, MRID 44256402.
NA=not applicable; ND=not detected; MMAD=mass median aerodynamic diameter; GSD=geometric standard deviation.

The analytical data indicate that the distribution of test article within the chamber was adequate, the absence of aerosol formation, and that the variance between nominal and actual exposure concentration to the animals was acceptable.

4. **Statistics:** Body weight, body weight change, and food consumption data, motor activity counts, fore-and hind-limb grip strength measurements, and landing foot splay measurements were analyzed statistically. Bartlett's test was performed to determine if the groups had equal variance. If the variances were equal, the data were analyzed by a one-way analysis of variance followed by the least significant difference technique to determine which means differed from the control. If the variances were unequal, the data were analyzed by the Kruskal-Wallis test followed by a summed rank test to determine differences between the means.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** During the 6-hour exposure, animals were observed once for clinical signs of toxicity. During the post-exposure period, animals were observed twice daily for mortality and morbidity. Detailed clinical observations were recorded twice pretest and weekly thereafter.

2. **Body weight:** Animals were weighed twice pretest, weekly during the study, and prior to sacrifice.

3. **Food consumption:** Food consumption was measured once pretest and weekly thereafter.

4. **Cholinesterase determination:** Cholinesterase activity was not measured.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** The FOB was conducted on all animals pretest, after exposure on day 1, and on days 8 and 15 of the observation period. Evaluations were performed by an observer blind to the animal's treatment group. Temperature (22-27°C), humidity (19-56%), noise level (41-57 dB), and illumination (19-58 footcandles) were controlled in the testing room. Animals were observed in the home cage, during handling, and in an open field for sensorimotor functions and coordination and autonomic functions. A list of the parameters assessed and the scoring criteria used were included. The duration of open field observation was not given. Grip strength was measured by a Grip Strength Meter, Columbus Instruments International Corp. For landing foot splay, a small dot of paint was applied to each hindpaw and the animal was dropped from a height of two feet; the distance between the marks left by the hindpaws was measured.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
X	Vocalization	X	Lacrimation* / chromodacryorrhea		Rearing+
	Convulsions*	X	Salivation*	X	Arousal/ gereral activity level*
	Tremors*	X	Piloerection* (open field)	X	Convulsions*
	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*		Palpebral closure*	X	Abnormal movements*
	Feces consistency		Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence* (open field)		Gait score*
	Touch response+		Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*				Backing
X	Pain response*				Time to first step
X	Pupil response*				
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension		Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. **Motor activity:** Following the FOB evaluation, motor activity was measured on all animals using an automated Photobeam Activity System, San Diego Instruments, Inc. each 60-minute session was divided into 12 five-minute intervals. Activity was measured as the number of beam breaks.

6. **Sacrifice and pathology:** At scheduled sacrifice, all animals were subjected to gross necropsy. Five animals/sex/group were anesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde/1% glutaraldehyde. The remaining 5 animals/sex/group were killed by exsanguination following carbon dioxide inhalation. From all perfused animals, the brain, spinal cord, and right sciatic, right tibial, and right sural nerves were preserved; gross lesions from all animals were also preserved. Brain and spinal cord were embedded in paraffin, sectioned at 5-6 µm, and stained with hematoxylin and eosin, Luxol Fast Blue, and Sevier-Munger stains. Peripheral nerves were embedded in glycol, sectioned at 2 µm, and stained with toluidine blue.

The CHECKED (X) tissues were evaluated from animals in the control and high-concentration groups.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Cerebral cortex	X	Sciatic nerve
X	Midbrain		
X	Hippocampus		
X	Basal ganglia		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling		Lumbar dorsal root ganglion
X	Lumbar swelling		Lumbar dorsal root fibers
X	Thoracic swelling		Lumbar ventral root fibers
	OTHER		Cervical dorsal root ganglion
	Gasserian Ganglion		Cervical dorsal root fibers
	Trigeminal nerves		Cervical ventral root fibers
	Optic nerve		
	Eyes		
	Gastrocnemius muscle		

7. **Positive controls:** Limited positive control data were included from a study conducted June-October, 1993. Results were given for FOB and motor activity assessments on male and female Sprague-Dawley rats administered 10 mg acrylamide/kg/day for 13 weeks. However, results from concurrent negative control animals were not included so that treatment-related effects could not be evaluated.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations.

2. **Mortality:** All animals survived to scheduled termination.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weight and body weight gain data are given in Table 3. For males and females, absolute body weight was similar between the treated and control groups throughout the study. Body weight gain was slightly decreased for the high-concentration groups during the post-exposure interval. Overall weight gain by the high-concentration males and females was 88% and 86%, respectively, of the control level.

TABLE 3. Body weight (g), body weight gain (g), and food consumption (g/kg/day)				
Endpoint	**0 ppm**	**100 ppm**	**300 ppm**	**500 ppm**
Males				
Body weight week -1	150.2 ± 4.8	150.7 ± 5.1	150.3 ± 4.4	150.0 ± 5.0
Body weight week 0	220.3 ± 5.2	218.0 ± 11.7	219.1 ± 8.4	214.3 ± 11.6
Body weight week 1	290.2 ± 11.5	284.8 ± 15.1	287.0 ± 15.7	275.4 ± 19.4
Body weight week 2	344.2 ± 12.4	337.0 ± 16.6	338.2 ± 21.8	322.9 ± 24.3
Weight gain week 0-1	69.9 ± 10.6	66.8 ± 5.2	67.9 ± 9.8	61.1 ± 11.0 (87)[a]
Weight gain week 0-2	123.9 ± 13.1	119.0 ± 7.2	119.1 ± 15.9	108.6 ± 16.3 (88)
Food consumption week 0	128.5 ± 5.9	129.0 ± 8.8	128.0 ± 6.2	127.1 ± 3.5
Food consumption week 2	85.6 ± 5.0	90.7 ± 4.0	87.1 ± 3.7	89.8 ± 6.3
Females				
Body weight week -1	113.0 ± 3.4	113.3 ± 3.7	112.9 ± 4.1	112.9 ± 3.6
Body weight week 0	158.1 ± 10.5	158.7 ± 5.7	153.0 ± 9.1	151.9 ± 12.0
Body weight week 1	189.5 ± 12.4	190.0 ± 7.0	187.1 ± 12.3	182.5 ± 12.8
Body weight week 2	218.5 ± 16.7	213.4 ± 11.3	212.9 ± 16.2	203.8 ± 13.0
Weight gain week 0-1	31.4 ± 6.6	31.3 ± 10.0	34.1 ± 8.9	30.6 ± 6.3
Weight gain week 0-2	60.4 ± 8.0	54.7 ± 14.3	59.9 ± 10.6	51.9 ± 10.2 (86)
Food consumption week 0	135.6 ± 7.0	139.9 ± 6.8	137.3 ± 7.3	138.9 ± 5.9
Food consumption week 2	97.7 ± 6.1	96.7 ± 5.7	96.7 ± 6.8	97.5 ± 8.3

Data taken from Appendix E, pp. 77-80, 83, and 84, MRID 44256402.
[a]Number in parentheses is percent of control, calculated by reviewer.
n=10/group

C. **FOOD CONSUMPTION**: Selected food consumption data are given in Table 3. No differences were observed between the treated and control groups of either sex.

D. **CHOLINESTERASE ACTIVITY**: Cholinesterase activity was not measured in this study.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings**: Selected FOB results after exposure on day 1 are given in Table 4. After exposure, an increased number of high-concentration males and females had drooping eyelids or half-closed eyes, a low arousal level, and no response to an approaching object. In the mid-concentration groups, increased incidences were observed for low arousal and no approach response in males, and for drooping eyelids in females. Slightly impaired locomotion was observed in two mid-concentration males, and in one and two high-concentration males and females, respectively, on day 1. In mid- and high-concentration males, some evidence of persistent effects were observed on day 8 and 15. On day 8 in the

control, low-, mid-, and high-concentration males, slightly impaired locomotion was seen in 1, 2, 5, and 4 animals, respectively, and low arousal was seen in 1, 3, 5, and 4, respectively. On day 15 in the control, low-, mid-, and high-concentration males, slightly impaired locomotion was observed in 1, 3, 5, and 7 animals, respectively, and low arousal was observed in 0, 3, 6, and 6, respectively. No effects were found on fore- and hind-limb grip strengths, landing foot splay, or reflex assessments.

TABLE 4. Functional observation battery results: incidence of findings on day 1

Observation	0 ppm	100 ppm	300 ppm	500 ppm
Males				
Eyelids drooping/half closed	1	1	0	5
Locomotion slightly impaired	0	0	2	1
Low arousal	0	2	5	9
Approach response - no reaction	1	2	6	6
Females				
Eyelids drooping/half closed	1	1	5	3
Locomotion slightly impaired	0	0	0	2
Low arousal	0	1	1	6
Approach response - no reaction	1	1	1	4

Data were extracted from Appendix G, pp. 369-408, MRID 44256402.
n=10

2. **Motor activity:** Total activity counts for each group on each testing day are given in Table 5. Activity level was markedly lower for high-concentration males and females compared with controls after exposure on day 1. Total activity on day 1 for high-concentration males and females was 50% and 55%, respectively, of the control group level. Activity by the mid-concentration males was 50% of the control level during the first 5-minute interval and total activity was 70% of the controls for the entire session. No other treatment-related effects were noted. Habituation was apparent in all groups on all days.

TABLE 5. Motor activity (total activity counts for session)				
Test day	**0 ppm**	**100 ppm**	**300 ppm**	**500 ppm**
Males				
Pre-test	734.4	814.1	682.9	604.3
Day 1	368.2	340.2	256.4 (30)[a]	184.5 (50)
Day 8	326	339.5	487.4	360.1
Day 15	935.6	898.2	992.1	849.7
Females				
Pre-test	577.7	571.4	661.8	546.9
Day 1	349.4	340	427.5	192.6 (45)
Day 8	373.1	332.8	474.5	566.6
Day 15	610.9	788.1	957.2	764.1

Data calculated by reviewer by adding session mean data, Appendix F, pp. 120-127, MRID 44256402.
[a]Number in parentheses is percent of control; calculated by reviewer. The total motor activity data lacks standard deviations because the study report provided only the sub session data.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No treatment-related lesions were found at necropsy.

2. **Brain weight:** Brain weight was not measured.

3. **Neuropathology:** No microscopic lesions were described for the brain, spinal column, or peripheral nerves from any control or high-concentration rat.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that a slight, transient decrease in the level of alertness was observed immediately after exposure in males and females exposed to 500 ppm and males exposed to 300 ppm. Decreases in motor activity in these groups were attributed to the decrease in alertness. Therefore, the NOEL was 100 ppm for males and 300 ppm for females.

B. REVIEWER COMMENTS: A single whole-body inhalation exposure to the test article resulted in systemic toxicity and evidence of neurotoxicity. Slight decreases in body weight gain were observed in animals exposed to the highest concentration although absolute body weight was not affected. However, in the absence of an effect on food consumption, lower weight gain by the exposed animals is considered toxicologically significant.

Neurotoxicity was evident as abnormalities recorded during the FOB and decreased motor activity. FOB findings in the mid- and high-concentration groups were consistent with sedation and were indicative of an effect on the central nervous system. In contrast, the

decreased motor activity should not be considered as due entirely to sedation (or "level of alertness") since results published in the open literature show that the test article affects both the central and peripheral nervous system, especially after multiple exposures. Additional evidence of an effect on the peripheral nervous system was seen as persistent impaired locomotion in mid- and high-concentration males.

Gross necropsy was unremarkable and no microscopic lesions were observed in the brain, spinal cord, or peripheral nerves.

The acute inhalation neurotoxicity LOAEL for ethylene oxide in Sprague-Dawley rats is 300 ppm based on FOB findings in males and females and impaired locomotion and reduced motor activity in males. The NOAEL is 100 ppm.

C. **STUDY DEFICIENCIES:** A minor deficiency was that the duration of open field observation was not stated. Positive control data were not included. Mean total motor activity was not reported and the study report included only subsession means for motor activity. However, these deviations would not have affected the outcome of the study.

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Conc. range ppm	Conc. tested ppm	NOAEL ppm	LOAEL ppm	Target organ(s)	Comments
042301	44256402	acute neurotox	rats	one exposure ; 6 hr	inhalation	whole body	100-500	0, 100, 300, 500	100	300	CNS, PNS	Toxicity

DATA EVALUATION RECORD

ETHYLENE OXIDE/042301
[OPPTS 870-6200 (§81-8)]

STUDY TYPE: SUBCHRONIC INHALATION NEUROTOXICITY STUDY IN RATS
MRIDs 44256401 (Range-Finding Study); 44359401 (Main Study)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task Order No. 79-2005

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: S. Ramasamy, Ph.D., D.A.B.T., M.P.H. **Signature:** Santhini Ramasamy
Reregistration Branch 4, Health Effects Division (7509C) **Date** 4.4.05
EPA Work Assignment Manager: G. Dannan, Ph.D. **Signature:** Ghazi Dannan
Registration Action Branch 3, Health Effects Division (7509C) **Date** 4/6/05

Template version 11/01

TXR#: 0052841

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity - Rats [OPPTS 870.6200 (§81-8)] OECD 424.

PC CODE: 042301 **DP BARCODE:** D235475
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Ethylene oxide (0.494-0.512%)

SYNONYMS: none

CITATION: Mandella, R.C. (1997) A 13-week inhalation neurotoxicity study of ethylene oxide (498-95A) in the rat via whole-body exposures with recovery. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study number 95-6099, August 12, 1997. MRID 44359401. Unpublished.

Mandella, R.C. (1997) A 4-week inhalation range-finding study of ethylene oxide (498-95A) in the rat via whole-body exposures. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study number 95-6098, March 21, 1997. MRID 44256401. Unpublished.

SPONSORS: AlliedSignal, Inc., 101 Columbia Road, Morristown, NJ 07962-1139 and ARC Chemical Division, Balchem Corporation, P.O. Box 180, Slate Hill, NY 10973

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 44359401), groups of fifteen Sprague-Dawley rats/sex were exposed to 0, 25, 50, 100, or 200 ppm Ethylene Oxide for six hours/day, five days/week for 14 weeks (at least 65 exposures) by whole body inhalation. Exposure concentrations were selected based on results of a range-finding study, MRID 44256401. To assess the reversibility of any observed effects, ten rats/sex/dose were observed during an additional 13-week recovery period. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on ten animals/sex/group pre-test, and during the exposure period (weeks 5, 9, and 14), and after the recovery period (weeks 27/28). Following at least 65 exposures, five animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Also, after the 13-week recovery period, five animals/sex/group were sacrificed and selected tissues preserved for neuropathological examination. The remaining five animals/sex/group were sacrificed and discarded. Of the

perfused animals, all control and high-concentration animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations. There were no deaths during the exposure period in any test group. One male exposed to 100 ppm was found dead during the recovery period (4 weeks after termination of exposure). Antemortem observations included lethargy, paleness, labored breathing, ano-genital staining, decreased fecal volume/no stool, and decreased food consumption.

Mean body weight was decreased approximately 8-13% ($p \leq 0.05$ or 0.01) in high-concentration males from week four until cessation of exposure in week 13. Mean body weight gain was decreased ($p \leq 0.05$ or 0.01) in high-concentration males during every week of the exposure period except week three; the decrease over weeks 1-13 was 16%. In high-concentration females, mean body weight was decreased approximately 8-10% ($p \leq 0.05$) from weeks 10 through 13, and mean body weight gain was decreased approximately 9% from weeks 6 through 13. The effects on body weight were reversible. The high-concentration males and females gained more weight than controls during the recovery period, and at week 26 mean body weight of high-concentration males was decreased only 3% compared to controls, and body weight gain of high-concentration females was increased 3.5% compared to controls. There were no treatment-related effects on body weight or body weight gain in either sex in the 25, 50, or 100 ppm groups. The only treatment-related effect on food consumption was an increase ($p \leq 0.05$) in food consumption in high-concentration males and females during the recovery period.

A 25% decrease ($p \leq 0.05$) in hindlimb grip strength in high-concentration females at the end of the exposure period was noted in the FOB assessment. No other treatment-related FOB effects were noted in either sex at any time point at any test concentration. There were no treatment-related effects on motor activity.

A malignant glioma was noted in the cerebral cortex of one high-concentration male following the recovery period. Mononuclear cell leukemia was noted in the male exposed to 100 ppm that died during week 4 of the recovery period. A hemangiosarcoma in the skin/subcutaneous tissue was noted in one 25 ppm male, and basal cell carcinoma of the skin was noted in one 50 ppm female and one 200 ppm female. Although there was no concentration-response relationship or common tumor type, these neoplasms were considered treatment-related because of the atypical presence of neoplasms in animals of this age and the lack of neoplasms in the control group.

Based on the results of this study, the NOAEL for ethylene oxide administered to Sprague-Dawley rats via whole body inhalation for 13-weeks (at least 65 exposures) is 100 ppm. The LOAEL is 200 ppm based on decreased body weight and body weight gain in males and females and decreased hindlimb grip strength in females.

Based on the data presented in this study, there is evidence of carcinogenicity in Sprague-Dawley rats exposed to ethylene oxide by inhalation during the thirteen week recovery period following at least 65 exposures.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. In the compliance statement it was noted that the study was not conducted according to GLP standards because empty test material cylinders were returned to the Sponsor during the course of the study to be refilled with test material and were not retained for the duration of the study, and because an archival sample of each lot of test material was not taken as required.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Ethylene oxide

Description:	colorless gas
Lot/Batch #:	a total of 171 cylinders were used in the subchronic study; one additional cylinder was used during the range-finding study; lot numbers are documented in the raw data
Purity:	0.493-0.512% mol.
CAS # of TGAI:	75-21-8
Structure:	

2. **Vehicle and/or positive control:** Clean air was used as the carrier and negative control. No positive control was used in this study.

3. **Test animals:**

Species:	Rat	
Strain:	Sprague-Dawley CD® [Crl: CD® BR]	
Age/weight at dosing:	6 weeks; M: 157-210 g; F: 123-172 g	
Source:	Charles River Laboratories, Kingston, New York	
Housing:	Animals were housed individually in suspended, stainless steel, wire mesh cages.	
Diet:	Certified Rodent Diet, No. 5002 was available *ad libitum* except during exposure.	
Water:	Mains water was available *ad libitum* except during exposure.	
Environmental conditions:	**Temperature:**	19-28°C
	Humidity:	17-81%
	Air changes:	not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	2 weeks	

B. STUDY DESIGN:

1. **In life dates:** Start: July 18, 1996; End: October 22, 1996

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 by a computerized random sort program so that body weight means were comparable. Animals were administered 6-hour exposures five days/week for 14 weeks (at least 65 exposures) by whole body inhalation and then maintained for two weeks. To assess the

reversibility of any observed effects, ten rats/sex/dose were observed during an additional 13-week recovery period. Concentration levels were based on the results of a range-finding study (MRID 44256401; see appendix). Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on ten animals/sex/group pre-test, and during the exposure period (weeks 5, 9, and 14), and after the recovery period (weeks 27/28). Following at least 65 exposures, five animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Also, after the 13-week recovery period, five animals/sex/group were scarificed and selected tissues preserved for neuropathological examination. The remaining five animals/sex/group were sacrificed and discarded. Of the perfused animals, all control and high-concentration animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

TABLE 1. Study design

Concentration (ppm)	Animals initially exposed		Neurobehavioral studies				Necropsy						Microscopic pathology					
			Pretest, Weeks 5, 9, & 14		Weeks 27/28 (recovery)		Week 14 (terminal)		Unscheduled deaths		Weeks 27/28 (recovery)		Week 14 (terminal)		Unscheduled deaths		Weeks 27/28 (recovery)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	15	15	10	10	10	10	5	5	0	0	10	10	5	5	–	–	6	5
25	15	15	10	10	10	10	5	5	0	0	10	10	0	0	–	–	6	5
50	15	15	10	10	10	10	5	5	0	0	10	10	1	1	–	–	6	5
100	15	15	10	10	10	10	5	5	1	0	9	10	0	0	1	–	5	6
200	15	15	10	10	10	10	5	5	0	0	10	10	5	5	–	–	6	6

Data taken from p. 15, MRID 44359401.

3. **Test substance preparation and analysis**: The exposure chambers were operated dynamically under slight negative pressure. Animals were individually housed in wire mesh cages within the 1000 L chamber. The test article was delivered through a regulator equipped with inlet and outlet backpressure gauges from the cylinder. Flow controller meter settings were selected in order to achieve target concentrations. For all chambers the air flow rate was 201-204 Lpm, the time for complete air change was 5 min, and the equilibrium time was 23 minutes. Animals remained in the chamber for 15 minutes after exposure to allow the chamber to clear using clean air only. Samples were taken four times during exposure to measure concentration using gas chromatography. Analytical concentrations were determined by comparing the concentration of test material detected to analytical standard concentrations of the test material. Prior to the start of the study, samples from five locations within the chamber were taken to assess distribution of the test article within the chamber. Particle size distribution measurements were performed once during each exposure using a TSI Aerodynamic Particle Sizer. Results of chamber monitoring are given in Table 2.

TABLE 2. Results of exposure chamber monitoring					
Endpoint	**0 ppm**	**25 ppm**	**50 ppm**	**100 ppm**	**200 ppm**
Distribution (range of concentration, ppm)	NA	21.8-28.1	47.8-57.2	92.8-112	185-244
Mean analytical concentration (ppm)	ND	25 ± 1.1	50 ± 1.8	101± 3.9	201 ± 7.7
Mean nominal concentration (ppm)	NA	27 ± 1.5	48 ± 1.5	96 ± 3.2	199 ± 5.4
Particle size MMAD (µm) GSD Total mass conc. (mg/m^3)	 1.99 ± 2.3 2.06 0.00660 ± 0.00819	 1.85 ± 1.6 2.32 0.00632 ± 0.00723	 1.59 ± 1.1 1.97 0.00567 ± 0.00669	 1.84 ± 1.8 2.07 0.00582 ± 0.00702	 2.25 ± 2.6 2.26 0.00629 ± 0.00719

Data taken from p. 33 and Appendix B, pp 70-91, MRID 44359401.
NA=not applicable; ND=not detected; MMAD=mass median aerodynamic diameter; GSD=geometric standard deviation.

The analytical data indicate that the distribution of test article within the chamber was adequate, no aerosol was formed, and the variance between nominal and actual exposure concentration was acceptable.

4. **Statistics:** Body weight, body weight change, and food consumption data, motor activity counts, fore-and hind-limb grip strength measurements, and landing foot splay measurements were analyzed statistically. Bartlett's test was performed to determine if the groups had equal variance. If the variances were equal, the data were analyzed by a one-way analysis of variance followed by the least significant difference technique to determine which means differed from the control. If the variances were unequal, the data were analyzed by the Kruskal-Wallis test followed by a summed rank test to determine differences between the means.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed once daily during exposure for clinical signs of toxicity. During the post-exposure period, animals were observed twice daily for mortality and morbidity. Detailed clinical observations were recorded twice pretest and weekly thereafter.

2. **Body weight:** Animals were weighed twice pretest, weekly during the study, and prior to sacrifice.

3. **Food consumption:** Food consumption was measured once pretest and weekly thereafter.

4. **Cholinesterase determination:** Cholinesterase activity was not measured.

5. Neurobehavioral assessment:

a. Functional observational battery (FOB): The FOB was conducted pretest, and during weeks 5, 9, 14, and 27/28. During the recovery period, FOB evaluations were performed during week 27; however, the fifth session occurred on the first day of week 28. Evaluations were performed by an observer blind to the animal's treatment group. Temperature (15-32°C), humidity (22-88%), noise level (54-63 dB), and illumination (12-73 footcandles) were controlled in the testing room. Animals were observed in the home cage, during handling, and in an open field for sensorimotor functions and coordination and autonomic functions. A list of the parameters assessed and the scoring criteria used were included. The duration of open field observation was not given. Grip strength was measured by a Grip Strength Meter, Columbus Instruments International Corp. For landing foot splay, a small dot of paint was applied to each hindpaw and the animal was dropped from a height of two feet; the distance between the marks left by the hindpaws was measured.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
X	Vocalization	X	Lacrimation* / chromodacryorrhea		Rearing+
	Convulsions*	X	Salivation*	X	Arousal/ gereral activity level*
	Tremors*	X	Piloerection* (open field)	X	Convulsions*
	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*		Palpebral closure*	X	Abnormal movements*
	Feces consistency		Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence* (open field)		Gait score*
	Touch response+		Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*				Backing
X	Pain response*				Time to first step
X	Pupil response*				
	Eyeblink response		PHYSIOLOGICAL OBSERVATIONS		NEUROMUSCULAR OBSERVATIONS
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension		Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. **Motor activity:** Following the FOB evaluation, motor activity was measured using an automated Photobeam Activity System, San Diego Instruments, Inc. Each 60-minute session was divided into 12 five-minute intervals. Activity was measured as the number of beam breaks per interval.

6. **Sacrifice and pathology:** At scheduled sacrifice, all animals were subjected to gross necropsy. Five animals/sex/group at terminal sacrifice and five animals/sex/group at recovery sacrifice were anesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde/1% glutaraldehyde. The remaining five animals/sex/group were killed by exsanguination following carbon dioxide inhalation. From all perfused animals, the brain, spinal cord, sciatic, tibial, and sural nerves, Gasserian ganglia, dorsal root ganglia, and dorsal and ventral root fibers were preserved; gross lesions from all animals were also preserved. Brain and spinal cord were embedded in paraffin, sectioned at 4-7 µm, and stained with hematoxylin and eosin, Luxol Fast Blue, and Sevier-Munger stains. Peripheral nerves, gasserian ganglion, and dorsal root ganglia and dorsal and ventral root fibers were embedded in glycol methacrylate, sectioned at 2 µm, and stained with toluidine blue.

   The CHECKED (X) tissues were evaluated from animals in the control and high-concentration groups.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Cerebral cortex	X	Sciatic nerve
X	Midbrain		
X	Hippocampus		
X	Basal ganglia		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
X	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
X	Trigeminal nerves	X	Cervical ventral root fibers
	Optic nerve		
	Eyes		
	Gastrocnemius muscle		

7. **Positive controls:** Positive control data were included from a study conducted October-December, 1996. Results were given for FOB and motor activity assessments on male and female Albino (VAF/Plus) rats administered chlorpromazine, D-amphetamine sulfate, or

phytostigmine by intraperitoneal injection for 5-6 weeks. These studies established that the procedures used for FOB and motor activity testing were valid.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** No treatment-related clinical signs of toxicity were observed in any animal during exposure, or during daily and weekly examinations.

2. **Mortality:** There were no deaths during the exposure period in any test group. One male exposed to 100 ppm was found dead during the recovery period (4 weeks after termination of exposure). Antemortem observations included lethargy, paleness, labored breathing, ano-genital staining, decreased fecal volume/no stool, and decreased food consumption. Necropsy suggested that death was due to malignant mononuclear cell leukemia.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Selected body weight and body weight gain data are summarized in Table 3. Mean body weight and body weight gain were decreased in high-concentration males and females; the effect was more pronounced in males. Mean body weight was decreased 8-13% ($p \leq 0.01$) in high-concentration males from week 4 until cessation of exposure in week 13. Mean body weight gain was decreased ($p \leq 0.05$ or 0.01) in high-concentration males during every week of the exposure period except week 3; the decrease over weeks 1-13 was 16%. In high-concentration females, mean body weight was decreased 9% ($p \leq 0.05$) from weeks 10 through 13, and mean body weight gain was decreased from weeks 6 through 13. The effects on body weight were reversible. The high-concentration males and females gained more weight than controls during the recovery period, and at week 26 mean body weight of high-concentration males was decreased only 3%, and mean body weight of high-concentration females was increased 3.5% compared to controls. There were no treatment-related effects on body weight or body weight gain in either sex in the 25, 50, or 100 ppm groups.

TABLE 3. Body weight (g), body weight gain (g), and food consumption (g/kg/day)					
Endpoint	**0 ppm**	**25 ppm**	**50 ppm**	**100 ppm**	**200 ppm**
Males					
Body weight week -1	120.6 ± 9.2	120.0 ± 8.6	120.4 ± 10.0	119.6 ± 8.8	119.6 ± 8.8
Body weight week 0	185.1 ± 10.6	183.9 ± 14.0	185.9 ± 10.1	184.6 ± 11.3	184.7 ± 11.7
Body weight week 1	271.7 ± 15.8	271.5 ± 19.5	275.3 ± 18.3	269.6 ± 14.6	262.5 ± 16.3
Body weight week 2	326.3 ± 20.1	327.7 ± 21.1	332.7 ± 23.0	324.8 ± 14.8	309.2 ± 19.4
Body weight week 4	406.1 ± 28.8	409.1 ± 23.9	409.3 ± 31.8	401.1 ± 21.4	373.3± 21.4** (8)[a]
Body weight week 9	513.2 ± 43.7	519.1 ± 40.3	517.9 ± 42.9	503.0 ± 39.4	446.3 ±22.7** (13)
Body weight week 13	564.5 ± 56.9	572.7 ± 47.1	572.8 ± 52.1	557.1 ± 41.3	494.1 ± 27.9** (12)
Body weight week 14	544.2 ± 43.4	567.9 ± 26.0	560.7 ± 48.7	562.7 ± 41.6	488.1 ± 30.6** (10)
Body weight week 16	578.5 ± 52.2	595.7 ± 34.4	586.0 ± 51.9	595.8 ± 39.8	524.1 ± 26.2* (9.4)
Body weight week 20	627.1 ± 58.1	648.5 ± 40.4	641.6 ± 63.2	688.6 ± 30.8	594.8 ± 24.2
Body weight week 26	675.6 ± 68.4	681.9 ± 38.6	695.5 ± 75.1	727.2 ± 32.0	655.1 ± 25.5
Weight gain week 0-13	369.7 ± 43.0	388.7 ± 39.6	381.0 ± 46.6	372.5 ± 41.2	309.4 ± 29.0** (16)
Weight gain week 0-26	491.4 ± 71.1	498.6 ± 34.9	507.2 ± 71.7	542.9 ± 33.3	472.9 ± 26.6
Food consumption week 0	144.7 ± 6.6	140.8 ± 5.1	145.4 ± 8.3	144.6 ± 6.3	146.3 ± 5.7
Food consumption week 2	96.8 ± 3.6	94.7 ± 2.3	98.2 ± 3.5	98.7 ± 4.0	99.2 ± 3.3
Food consumption week 4	76.1 ± 2.3	74.8 ± 4.5	77.3 ± 2.4	75.7 ± 4.0	78.5 ± 3.5
Food consumption week 13	51.7 ± 3.8	49.2 ± 3.5	50.7 ± 3.3	52.0 ± 3.5	58.1 ± 3.2** (12)
Food consumption week 20	48.3 ± 2.2	47.8 ± 2.8	50.7 ± 2.9	47.1 ± 3.1	53.9 ± 4.2** (12)
Food consumption week 26	43.5 ± 2.8	41.0 ± 5.8	45.5 ± 2.3	41.8 ± 2.0	44.3 ± 2.9
Females					
Body weight week -1	104.1 ± 6.3	104.1 ± 6.8	103.9 ± 6.8	103.9 ± 6.7	104.1 ± 7.2
Body weight week 0	148.5 ± 6.7	146.2 ± 11.1	145.5 ± 7.5	149.5 ± 9.9	148.3 ± 8.7
Body weight week 1	188.0 ± 10.4	188.1 ± 14.6	186.1 ± 10.5	193.0 ± 11.4	187.5 ± 14.8
Body weight week 2	211.6 ± 11.8	209.8 ± 15.2	208.5 ± 8.5	218.0 ± 15.8	206.9 ± 16.7
Body weight week 4	251.1 ± 16.5	245.1 ± 18.7	242.5 ± 11.8	257.5 ± 20.7	240.0 ± 24.3
Body weight week 9	290.9 ± 22.1	286.4 ± 27.5	273.7 ± 16.8	293.4 ± 28.8	271.4 ± 25.8
Body weight week 13	309.4 ± 29.3	300.6 ± 26.1	287.8 ± 21.7	313.3 ± 31.6	282.0 ± 29.9* (8.9)
Body weight week 14	305.4 ± 29.6	286.7 ± 26.4	283.3 ± 18.4	303.0 ± 40.3	278.6 ± 21.4
Body weight week 16	311.0 ± 36.0	298.5 ± 31.4	290.6 ± 21.6	314.6 ± 36.6	290.1 ± 27.4
Body weight week 20	325.6 ± 38.7	316.5 ± 31.1	310.8 ± 23.5	339.3 ± 43.2	328.0 ± 31.7
Body weight week 26	340.4 ± 47.6	330.0 ± 34.4	326.8 ± 34.9	350.3 ± 46.2	352.2 ± 38.4
Weight gain week 0-13	160.9 ± 25.8	154.4 ± 18.6	142.3 ± 19.8	163.9 ± 24.0	133.7 ± 23.8** (17)

Endpoint	0 ppm	25 ppm	50 ppm	100 ppm	200 ppm
Weight gain week 0-26	193.1 ± 44.3	187.0 ± 31.4	183.6 ± 33.2	202.7 ± 36.9	205.2 ± 37.2
Food consumption week 0	142.6 ± 8.7	138.4 ± 8.2	139.3 ± 9.9	138.9 ± 9.0	141.6 ± 9.3
Food consumption week 2	101.8 ± 2.9	98.0 ± 8.6	101.5 ± 4.5	101.9 ± 6.4	108.3 ± 10.5
Food consumption week 4	87.6 ± 4.2	82.7 ± 5.4*	85.7 ± 3.7	83.6 ± 3.7	90.9 ± 7.0
Food consumption week 13	65.0 ± 3.6	65.1 ± 4.7	66.0 ± 4.2	66.4 ± 3.6	72.0 ± 6.3** (11)
Food consumption week 20	61.6 ± 5.0	65.6 ± 4.9	67.8 ± 3.1	61.3 ± 5.6	71.1 ± 8.1* (15)
Food consumption week 26	57.8 ± 5.9	61.6 ± 9.7	59.9 ± 5.9	55.3 ± 4.7	56.0 ± 6.3

Data taken from Appendix E, pp. 130-154, MRID 44359401.
*p≤0.05; **p≤0.01.
'Number in parentheses is percent decrease (body weight/body weight gain) or increase (food consumption) compared to control, calculated by reviewer.
n=10-15/group

C. **FOOD CONSUMPTION**: Selected food consumption data are given in Table 3. The statistical increases (p≤0.05 or 0.01) in food consumption observed in high-concentration males and females during the exposure period were attributed to decreased body weight because food consumption expressed as grams/interval was similar between high-concentration and control groups. However, the increased (p≤0.05) food consumption noted in high-concentration males and females during the recovery period was attributed to an actual increase in food consumption because consumption expressed as grams/interval differed between control and high-concentration groups. No differences were observed between the 25, 50, or 100 ppm groups and control groups of either sex.

D. **CHOLINESTERASE ACTIVITY**: Cholinesterase activity was not measured in this study.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings**: At week 14 (end of exposure period), there was a 25% decrease (p≤0.05) of hindlimb grip strength in high-concentration females only. This effect is considered treatment-related; however, the study authors did not consider this observation to be a sign of frank neurotoxicity because all other measures of neurological function (locomotion, gait, landing foot splay, air-righting reflex) were comparable to controls. No other treatment-related FOB effects were noted in either sex at any time point at any test concentration. Moderate difficulty in handling was noted in males and females at week 14 and low arousal was noted in some males after the recovery period. However, these observations were noted in control and treated groups at similar incidences and are considered incidental to treatment. Landing foot splay was decreased (p≤0.05) in females in the 100 ppm group at week 14 and in females in the 100 and 200 ppm groups at recovery. However, these observations are attributed to a decrease (compared to previous trials) in landing foot splay in controls and are not considered treatment-related. A decrease in landing foot splay was also noted in 50 ppm males after the recovery period; however, in the absence of a concentration-response relationship, this effect is also considered incidental to treatment.

2. **Motor activity:** Mean total activity counts for each group on each testing day are given in Table 4. No treatment-related effects were noted, and habituation was apparent in all groups on all days.

TABLE 4. Motor activity (mean total activity counts for session)

Test day	0 ppm	25 ppm	50 ppm	100 ppm	200 ppm
Males					
Pre-test	805.5	820.9	690.6	748.2	802.9
Week 5	1187	1228	1220	1002	1429
Week 9	1056	1166	958	1110	942
Week 14	946.5	931.9	870.0	912.4	922.1
Recovery	525.1	544.2	576.0	499.3	646.9
Females					
Pre-test	686.2	722.4	568.3	905.5	843.3
Week 5	770.8	879.5	753.4	977.9	831.9
Week 9	619.5	673.1	689.9	748.6	562.6
Week 14	438.5	513.0	565.2	589.0	512.6
Recovery	576.7	542.1	602.7	594.8	705.0

Data calculated by reviewer by adding session mean data, Appendix F, pp. 448-457, MRID 44359401.
[a]Number in parentheses is percent of control; calculated by reviewer. The total motor activity data lacks standard deviations because the study report provided only the sub session data.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related findings at the end of the exposure period. At the end of the recovery period, masses were noted in three rats: one in the cervical region of a 25 ppm male, one on the trunk of a 50 ppm female, and one on the trunk of a 200 ppm female. One male in the 100 ppm group died during week 4 of the recovery period. Necropsy of this animal indicated a severely enlarged liver and spleen. These findings correlated with microscopic findings of malignant neoplasms (Section F.3).

2. **Brain weight:** Brain weight was not measured.

3. **Neuropathology/ Microscopic findings:**

Microscopic examination of the spinal cord, peripheral nerves, gasserian and dorsal root ganglia, and dorsal and ventral root fibers of the high-concentration animals immediately after the exposure period and after the recovery period revealed no treatment-related effects. There were no brain lesions immediately after exposure; however, a malignant glioma was noted in the cerebral cortex of one high-concentration male following the recovery period. Because of this finding, brains from low- and mid-exposure animals were also examined after the recovery period; no other brain lesions were identified.

Malignant neoplasms were also noted in other rats during the evaluation of gross lesions. Mononuclear cell leukemia was noted in the male exposed to100 ppm that died during week 4 of the recovery period. A hemangiosarcoma in the skin/subcutaneous tissue was noted in one 25 ppm male, and basal cell carcinoma of the skin was noted in one 50 ppm female and one 200 ppm female.

Although there was no concentration-response relationship or common tumor type, the five neoplasms, distributed across all treatment-groups, were considered treatment-related because of the atypical presence of neoplasms in animals of this age and the lack of neoplasms in the control group.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that there were no neurobehavioral effects associated with whole body exposures of Sprague-Dawley rats to 25, 50, 100, or 200 ppm ethylene oxide for at least 65 exposures. Therefore, the neurotoxicity NOEL was 200 ppm.

The author also concluded that the malignant neoplasms found during or after the recovery period were treatment-related, even though there was no concentration-response relationship or common tumor type. These neoplasms were considered treatment-related because of the atypical presence of neoplasms in animals of this age and the lack of neoplasms in the control group. Based on carginogenicity, the author concluded that a NOEL was not determined.

B. REVIEWER COMMENTS: Subchronic whole-body inhalation exposure to the test article resulted in systemic toxicity (as evidenced by decreased body weight and body weight gain). The reviewer's conclusions are different from that of investigator with regard to neurotoxic effects. The neurotoxic effects as evidenced by decreased hindlimb grip strength in females was considered treatment related.

Lesions identified at gross necropsy had histological correlates of malignancy. Even in the absence of a concentration-response relationship and common tumor type, these malignant neoplasms are considered treatment-related because of the age of the animals and the lack of neoplasms in the control group.

Based on the results of this study, the NOAEL for ethylene oxide administered to Sprague-Dawley rats via whole body inhalation for 13-weeks is 100 ppm. The LOAEL is 200 ppm based on decreased body weight and body weight gain in males and females and decreased hindlimb grip strength in females.

Based on the data presented in this study, there is evidence of carcinogenicity in Sprague-Dawley rats exposed to ethylene oxide by inhalation for at least 65 exposures.

C. STUDY DEFICIENCIES: A minor deficiency was that the duration of open field observation was not stated. Also, mean total motor activity was not reported and the study

report included only subsession means for motor activity. However, these deviations would not have affected the outcome of the study.

DATA FOR ENTRY INTO ISIS

Subchronic Neurotoxicity Study - rats (870.6200)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Conc. range ppm	Conc. tested ppm	NOAEL ppm	LOAEL ppm	Target organ(s)	Comments
042301	44359401	subchronic neurotox	rats	6 hr/day, 5 days/week, 14 weeks	inhalation	whole body	25-200	0, 25, 50, 100, 200	100	200	Systemic and nervous system	Decreased BW and BW gain and decreased hindlimb grip strength

APPENDIX

MRID 44256401

STUDY TYPE: 4-Week Inhalation Range-Finding- Rat

PC CODE: 042301 **DP BARCODE:** D235475
MRID No.: 44256401

TEST MATERIAL (PURITY): Ethylene Oxide (0.480%- 0.520% in Nitrogen)

SYNONYMS: None

CITATION: Mandella, R.C. (1997) A 4-week inhalation range-finding study of ethylene oxide (498-95A) in the rat via whole-body exposures. Huntingdon Life Sciences, Mettlers Road, East Millstone, New Jersey. Laboratory study number 95-6098, March 21, 1997. MRID 44256401. Unpublished.

SPONSORS: AlliedSignal, Inc., 101 Columbia Road, Morristown, NJ 07962-1139 and ARC Chemical Division, Balchem Corporation, P.O. Box 180, Slate Hill, NY 10973

EXECUTIVE SUMMARY: In a range-finding study (MRID 44256401), groups of five Sprague-Dawley rats/sex were exposed to target concentrations of 0, 100, 300, 400, or 500 ppm Ethylene Oxide for six hours/day, five days/week for 4 weeks by whole body inhalation. The study was designed to assess the inhalation toxicity of ethylene oxide and to establish exposure concentrations for longer-term studies. Mean analytical exposure concentrations were 0, 98.4 ± 3.76, 300 ± 15, 397 ± 23, and 501 ±12 ppm. The mean nominal concentrations were 0, 109 ± 10.7, 292 ± 19, 382, ± 24, and 506 ± 11 ppm. Particle size distribution measurements suggested the absence of any aerosol formation.

One 500 ppm female was found dead on day 18. Antemortem clinical signs included irregular gait, labored breathing, paleness, lethargy, emaciation, anogenital stains, black/brown stains on the snout, unthrifty coat, decreased food consumption, and decreased fecal volume/no stool. All other rats survived to terminal sacrifice. Irregular gait and decreased fecal volume were observed in all animals exposed to 500 ppm. Some animals in the 500 ppm group also exhibited lethargy, prostration, emaciation, yellow angenital staining, moist rales, labored breathing, black/brown stains on the snout, paleness, emaciation, and decreased food consumption.

Decreased ($p \leq 0.05$ or 0.01) mean body weight and body weight gain were observed in animals exposed to 300, 400, or 500 ppm. At week 4, mean body weight was decreased 12.1%, 23.8%, and 42.4% in males and 14.4%, 20.1%, and 40.6% in females in the 300, 400, and 500 ppm groups, respectively. Food consumption was decreased ($p \leq 0.01$) in 500 ppm males in weeks 1 (18%) and 3 (12%) and in 500 ppm females in week 1 (15%).

Hindlimb grip strength was decreased ($p \leq 0.05$ or 0.01) at 300, 400, and 500 ppm in both males and females at weeks 3 and 4 in all trials. In the 500 ppm group, decreases averaged 53% and 60% at week 3 and 76% and 86% at week 4 for males and females, respectively. In the 400 ppm

group, decreases averaged 31% in males and 38% in females at week 3 and 48% for both sexes at week 4. In the 300 ppm group, decreases averaged 27% in males and 23% in females at week 3 and 36% for males and 22% for females at week 4. Landing foot splay was also decreased ($p \leq 0.05$ or 0.01) in both sexes at weeks 3 and 4 in the 400 and 500 ppm groups. In the 500 ppm group, decreases averaged 65% and 64% at week 3 and 68% and 72% at week 4 for males and females, respectively. In the 400 ppm group, decreases averaged 33% in males and 31% in females at week 3 and 42% for males and 29% for females at week 4.

Decreased absolute brain weight was noted in males exposed to 400 (6.8%; $p \leq 0.05$) or 500 ppm (8.9%; $p \leq 0.01$). No brain weight effects were noted in any females or in males exposed to 100 or 300 ppm. No treatment-related macroscopic lesions were noted at necropsy. Treatment-related microscopic lesions were noted in all males and females from the 500 ppm group and included minimal to slight vacuolation of the white matter of the thalamus and medulla oblongata. No similar microscopic lesions were noted in the control animals.

Based on the results of this study, the NOAEL for ethylene oxide administered to Sprague-Dawley rats via whole body inhalation for 4-weeks is 100 ppm. The LOAEL is 300 ppm based on decreased body weight and body weight gain and decreased hindlimb grip strength.

13544

R106909

Chemical:	**Ethylene oxide**
PC Code:	**042301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**04/07/2005**
File ID:	**TX0052841**
Accession Number:	**412-05-0094**

HED Records Reference Center
04/29/2005